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(71) Applicant: **MICROPROBE CORPORATION**
1725-220th Street S.E. Suite 104
Bothell, WA 98021(US)

(72) Inventor: **Van Ness, Jeffrey**
10701-189th Street N.E.
Bothell, Washington 98011(US)
Inventor: **Tabone, John C.**
12117 N.E. 166th Place
Bothell, Washington 98011(US)
Inventor: **Petrie, Charles E.**
18459 N.E. 166th Place
Woodinville, Washington 98072(US)
Inventor: **Vermeulen, Nicolaas M.J.**
19334-196th Avenue, N.E.
Woodinville, Washington 98072(US)

(74) Representative: **Thomson, Paul Anthony et al**
Potts, Kerr & Co. 15, Hamilton Square
Birkenhead Merseyside L41 6BR(GB)

(54) Solid supports for nucleic acid hybridization assays and methods for covalently immobilizing oligonucleotides.

(57) Compositions and methods for covalently immobilizing an oligonucleotide onto a polymer-coated bead or similar structure are described. Specifically, the polymer-coated bead or similar structure possesses a large number of activatable moieties, preferably primary and secondary amines. An oligonucleotide is activated with a monofunctional or multi functional reagent, preferably the homotrifunctional reagent cyanuric chloride. The resultant covalently immobilized oligonucleotides on the beads or similar structures can serve as nucleic acid probes on solid supports, and hybridization assays can be conducted wherein specific target nucleic acids are detected in complex biological samples. The beads or similar structures can be employed free in solution, such as in a microtiter well format; in a flow-through format, such as in a column; or in a dipstick, including a multisite indicator card wherein multiple beads possessing oligonucleotides with different sequences or specificities can be closely aligned whereby a multiplicity of pathogens can be detected in a single biological sample. Additionally, dichlorotriazine oligonucleotides and processes for activating oligonucleotides by treatment with cyanuric chloride are included in the present invention.

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immobilized capture nucleic acid can be increased approximately 20-fold on an apparent surface area basis; third, greater ease of manufacturing exists; fourth, the bead possesses covalently immobilized, capture nucleic acid sequences (oligonucleotides) and can withstand denaturation temperatures in excess of 90°C for 10 or more minutes; and, finally, a multisite dipstick can be constructed, leading to miniaturization of a detection device. All of these advantages contribute to greater sensitivities when, for example, a sandwich assay format is used.

Dipsticks are also included in the present invention. These dipsticks comprise a nonporous solid support and a means for attaching the beads or similar structures discussed above. Additionally, multiple beads possessing oligonucleotides with different sequences or specificities can be closely aligned on a multisite dipstick, giving rise to an indicator card that can detect a multiplicity of pathogens in a single biological example, for example, to identify bacterial and viral agents.

The use of beads or similar structures in the dipstick format achieves a significant decrease in nonspecific background levels of signal systems because of the simple pressure fit by which the bead or similar structure is placed in the dipstick, as compared with membrane supports and the like, which necessarily must be sandwiched between two supports or glued or attached in place.

A further understanding of the nature and advantages of the present invention may be realized by reference to the remaining portions of the specification and to the drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 is a configuration for a dipstick of the present invention;

Fig. 2 is a multisite indicator card that can be used for periodontitis diagnosis; and

Fig. 3 are high performance liquid chromatography (HPLC) profiles showing that cyanuric chloride reacts selectively with the 5'-tethered amine of an oligonucleotide, and not with sugars or bases thereof.

DETAILED DESCRIPTION OF THE INVENTION

The present invention includes compositions useful in nucleic acid hybridization assays. These compositions comprise a dichlorotriazine oligonucleotide and other activated oligonucleotides covalently attached to a polymer-coated bead or similar structure.

Additionally, processes for covalently immobilizing an oligonucleotide on a nylon solid support, preferably a bead, are included. In general, these processes comprise the steps of treating a nylon solid support with an alkylating agent; reacting the treated solid support with an amine-containing polymer, whereby the polymer covalently coats the solid support; activating an oligonucleotide with a monofunctional or multifunctional reagent, preferably the homotrifunctional reagent, cyanuric chloride (i.e., 2,4,6-trichlorotriazine); conjugating the activated oligonucleotide and the polymer-coated solid support. The unreacted amines are then blocked by acylatation to impart the proper surface charge to the solid support surface.

The term "solid support" refers to any surface that is transferable from solution to solution or forms a structure for conducting oligonucleotide-based assays, and includes beads, membranes, microtiter wells, strings, plastic strips, or any surface onto which nucleic acid probes may be immobilized.

As used herein, "bead" encompasses any type of solid or hollow sphere, ball, bearing, cylinder, or other similar configuration composed of plastic, ceramic, metal, or polymeric material onto which a nucleic acid can be covalently immobilized. As such, the term also includes nylon string or strings. Preferably, a nylon bead that is spherical in shape is employed in the present compositions, and a preferred diameter range for such beads is from about 0.01 inch to about 0.5 inch, more preferably from about 0.06 inch to about 0.09 inch (corresponding to commercially available 3/32 inch nylon beads), and most preferably about 0.09 inch (corresponding to commercially available 3/32 inch nylon beads). Additionally, it is preferred that the nylon beads are unpolished or, if polished, roughened before treating with an alkylating agent.

In the present invention, a nylon bead or beads, or any composition or structure of nylon, is first derived (prepared) by treating the bead with an alkylating agent. Alkylating agents used in this manner react with amides present in the nylon to form a reactive imidate ester.

Preferred alkylating agents include, but are not limited to, dialkyl sulfates, alkyl triflates, alkyldiphenyl sulfonium salts, alkyl perchlorates, and, preferably, trialkyloxonium salts. The latter includes the lower alkyl salts, trimethyloxonium and triethyloxonium salts. The salt counterion can be selected from the group consisting of hexachloroantimonate, hexafluorophosphate, and tetrafluoroborate, with the last named counterion being preferred; however, other salt counterions can also be used and will be apparent to one skilled in the relevant art.

The selection of a solvent for the alkylating agent is important for the present invention. A solvent

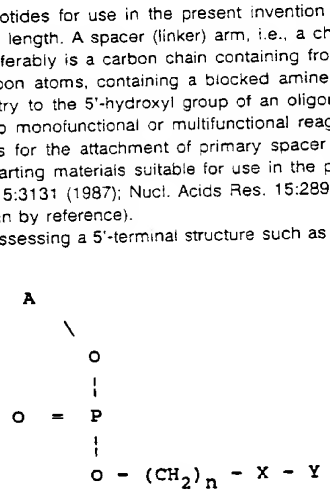
Bead functionality:	Polymer functionality:	Coupling reagent:
Amine	Amine	Cyanuric chloride
Amine	$-COO^-$	Carbodiimide
Amine	-SH	Heterobifunctional
$-COO^-$	Amine	Carbodiimide
-OH	Amine	Cyanuric chloride

The polymer-coated solid supports are then conjugated with activated oligonucleotides using similar or identical chemistries to those described above. As used herein, oligonucleotides refer to short nucleic acid sequences that are approximately 16 to 100 bases in length. Such oligonucleotides can be used as capture probes in hybridization assays and are preferably chemically synthesized using commercially available methods and equipment. For example, the solid phase phosphoramidite method can be used to produce short probes of between 15 and 50 bases having a molecular weight of less than 16,000 daltons. For the synthesis of oligonucleotides, see Caruthers, et al., Cold Spring Harbour Symp. Quant. Biol., 47:411-418 (1982); Adams, et al., J. Am. Chem. Soc., 105:661 (1983) (both are incorporated herein by reference).

When synthesizing an oligonucleotide probe for a specific target nucleic acid, the choice of nucleotide sequence will determine the specificity of the test. For example, by comparing DNA sequences from several bacterial isolates, one can select a sequence for bacterial detection that is either type-specific or genus-specific. Comparisons of DNA regions and sequences can be achieved using commercially available computer programs.

The preferred capture oligonucleotides for use in the present invention are synthetic oligonucleotides from about 20 to about 100 bases in length. A spacer (linker) arm, i.e., a chemical moiety that extends or links other chemical groups, and preferably is a carbon chain containing from about 2 to about 12 carbon atoms, more preferably about 6 carbon atoms, containing a blocked amine group can be coupled during synthesis using conventional chemistry to the 5'-hydroxyl group of an oligonucleotide. A primary amine is the preferred group for attachment to monofunctional or multifunctional reagents, and its attachment via a hexyl arm is preferred. The reagents for the attachment of primary spacer arms terminating in a primary amine are commercially available. starting materials suitable for use in the present invention are described in PCT 86/01290; Nucl. Acids Res. 15:3131 (1987); Nucl. Acids Res. 15:2891 (1987); and Nucl. Acids Res. 14:7985 (1986) (all incorporated herein by reference).

Preferably, an oligonucleotide possessing a 5'-terminal structure such as



is employed wherein, as a spacer arm, n is 2-12 inclusive, preferably 6; X is -NH- or $-NHC{:}O(CH_2)_mNH-$, preferably -NH-; wherein m is 2-12, inclusive; Y is a 4,6-dichlorotriazine (preferred) or thiol (sulfhydryl) reactive moiety; and A is an oligonucleotide, ranging from between about 9-50 bases, preferably between about 15-30 bases, with only the 5'-hydroxyl requiring modification for attachment.

Alternatively, an oligonucleotide can be modified at the 3'-end with a spacer arm containing a blocked amine group. This can be accomplished by conducting DNA synthesis on a solid support containing a conjugated ribonucleotide. After removal from the solid support, a DNA oligonucleotide is obtained that

colored labeles, and immunogenic labels; etc. Dichlorotriazine oligonucleotides are included in the present invention, as are processes for activating oligonucleotides with cyanuric chloride to form dichlorotriazine oligonucleotides as discussed below.

The unreacted cyanuric chloride can be removed by exclusion chromatography or ultrafiltration, and the bead and derived oligonucleotide conjugated wherein they are mixed together and incubated at, preferably 20 to 50°C for 1 to 24 hours. The residual (unreacted) amines on the bead surface can be blocked (capped) with an agent, such as succinic anhydride, preferably in N-methyl pyrrolidone in the presence of an appropriate base such as sodium borate, to render the surface compatible (negatively charged) for nucleic acid hybridization. Such blocking of amines occurs through an acylating reaction or reaction of amines with an activated ester, resulting in a nonactivatable moiety. It should be noted that the ability exists for the bead surface to chemically derived such that a positive, negative, or neutral charge can be placed on the bead.

In addition, 4,6-dichlorotriazine moieties can be replaced with thiol reactive substituents and, preferably, spacer arms are present. The preferred spacer arms are derived from thiol reactive substituents linked to a 5'-tethered nucleophilic amine and are of the same formula as listed above with the exception that Y in the formula above is a thiol reactive moiety. A preferred thiol reactive moiety has a reactive group of either an α halo-acyl or an α, β-unsaturated carbonyl. The most preferred thiol reactive moieties are selected from the group consisting of haloacetamidobenzoyl and 4-(N-maleimidomethyl)- cyclohexane-1-carbonyl.

A polymeric structure as described above wherein the polymeric structure is derived with thiol (sulfhydryl) containing moieties can be used in the present invention. The actual structure of the thiol containing moieties is non-critical as long as the thiol group or groups are available to react with thiol reactant moieties. The thiol chemistries in this instance are replacements for cyanuric chloride chemistries.

The present invention also includes dipsticks having utility in nucleic acid hybridizations and comprising a nonporous solid support and a means for attaching a bead. Nonporous solid supports are known in the art, and the present invention is concerned with attaching a bead to a dipstick. An example of bead attachment is the presence of a perforation or perforations (or a depression or depressions) in the dipstick wherein beads can be attached. Preferably, perforations are employed and the beads are attached through a pressure fit with the circumference of the hole. Such a pressure fit can occur if, for example, the circumference of the perforation (or depression) is slightly less than the cirumference of the bead so that the bead is pressed in place.

Preferred beads are as listed above, and may be covalently attached, either directly or through a spacer arm, to activated oligonucleotides of the same or different sequence per a given bead, as also described above.

The dipstick can contain more than one bead, preferably from about two to ten, each in their own hole, and more preferably, situated in a row along one edge of the dipstick. Such a dipstick can function as an indicator card, wherein multiple beads covalently attached to oligonucleotides with different sequences or specificities can be closely aligned on a multisite dipstick, which can detect a multiplicity of pathogens in a single biological sample. A particular bead may contain oligonucleotides with more than one nucleic acid sequence, for example, sequences from related organisms, or a bead may only contain oligonucleotides with a given nucleic acid sequence.

Numerous organisms and cell types, including pathogenic and nonpathogenic entitites, can be detected in this manner from a variety of biological sample types. Organisms include bacteria and viruses as well as other microorganisms, and cell types include, for example, those involved in inherited diseases and metabolic disorders. Many other detection applications will be apparent to one of ordinary skill in the art. For example, purported causative bacterial agents of periodontitis, such as Actinobacillus actinomycetemcomitans, Bacteroides gingivalis, Bacteroides forsythus, Bacteroides intermedius, Eikenella corrodens, Fusobacterum nucleatum, and Wolinella recta, can be identified.

It will be obvious to one of ordinary skill in the art that, although the present invention is described in terms of nucleic acid hybridization assays, many other uses for these dipsticks are possible. Any member of a ligand pair can be attached to beads in the dipstick, and the dipstick can then be used to identify the corresponding ligand member. For example, antigens or antibodies could be attached to beads, as described above, in a dipstick and then their corresponding antibodies or antigens, respectively, could be identified. In a similar manner, biotin and streptavidin can be used.

Furthermore, the present invention also includes processes for nucleic acid detection wherein a composition comprising a polymer-coated bead, preferably having activatable amine groups, covalently attached to an activated oligonucleotide is contacted with a target nucleic acid under suitable conditions for hybridization and the hybridized product is detected. Such processes can occur in a microtiter well, a flow-through column, and using a dipstick, as described above.

Target nucleic acid is usually a polynucleotide with an average length from about 20 to about 20,000

CAP buffer is 0.1 M NaCitrate pH = 6.5 and 0.2 M NaPhosphate.

The fluorescent substrate for alkaline phosphatase is 0.02 mM 4-methyl-umbelliferone phosphate, 0.05 M Tris pH = 9.5, 1 mM $MgCl_2$, 0.5 mM $ZnCl_2$.

The chemiluminescent substrate for alkaline phosphatase was a pre-prepared cocktail from Lumigen, Inc. (Detroit, MI).

Oligonucleotide sequences:

```
Bg1: 5'-XCAATACTCGTATCGCCCGTTATTC-3'
Aa004: 5'-XACCCATCTCTGACTTCTTCTTCGG-3'
Bg016: 5'-XTACTCGTATCGCCCGTTATTCCCG-3'
Ek007: 5'-XAAAAGTGGTATTAGCACTTCCCTT-3'
PA005: 5'-XGACATACCTTCCACCATCTGCAAG-3'
PA505: 5'-XCTTGCAGATGGTGGAAGGTATCTC-3'

UP9A: 5'-XCTGCTGCCTCCCGTAGGAGT-3'
UP007: 5'-XGTATTACCGCGGCTGCTG-3'
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Poly(ethyleneimine) was purchased from Polysciences (Warrington, PA).

Burnished or unpolished nylon beads were purchased from Precision Ball Company (Chicago, IL) and The Hoover Group (Sault St. Marie, MI.)

Triethyloxonium tetrafluoroborate, hexanediamine, phenylenediamine, succinic anhydride and N-methyl-pyrrolidinone (N-methyl-pyrrolidone, m-pyrol) were purchased from Aldrich Chemical (Milwaukee, IL).

N-succinimidyl 4-(iodoacetamido)-benzoate (SIAB) and Tween 20 was purchased from Pierce (Rockford, IL).

Guanidium isothioscyanate (GuSCN) was purchased form Kodak (Rochester, NY).

Nylon membrane, Nytran™, was purchased from Schleicher & Schuell, (Keene, NH).

The di- and triamines EDR-148, ED-400, ED-6000, and T-3000 were a gift from Texaco Chemical Company, (Houston, TX).

Procedures

Oligonucleotide synthesis:

Oligonucleotides complementary to regions conserved or hypervariable regions of the 16S-ribosomal RNA of either Actinobacillus actinomycetemcomitans (Aa), Bacteroides gingivalis (Bg), Bacteroides intermedius (Bi), Eikenella corrodens (Ek), Fusobacterium nucleatum (Fn), or Wolinella recta (Wr) were synthesized using phosphoramidite chemistry on either an ABI 380B or a Milligen 7500 automated DNA synthesizer. The oligonucleotides were prepared using the standard phosphoramidite chemistry supplied by the vendor or the H-phosphonate chemistry. Appropriately blocked dA, dG, dC, and T phosphoramidites are commercially available in these forms, and synthetic nucleosides may readily be converted to the appropriate form. Oligonucleotides were purified by adaptations of standard methods. Oligonucleotides with 5'-trityl groups were chromatographed on HPLC using a 12 µm, 300 Å Rainin (Woburn, MA) Dynamax C-8 4.2x250 mm reverse phase column using a gradient of 15% to 55% MeCN in 0.1 N $Et_3NH^+OAc^-$, pH 7.0, over 20 min. When detritylation was performed, the oligonucleotides were further purified by gel exclusion chromatography. Analytical checks for the quality of the oligonucleotides were conducted with a Toso-Haas DEAE-NPR column at alkaline pH and by polyacrylamide gel electrophoresis (PAGE).

Preparation of the polymer-coated nylon bead:

25,000 3/32 inch diameter unpolished nylon beads were placed in a flask containing 1800 ml of 100% anhydrous n-methyl-pyrrolidinone and mixed for 5 minutes at ambient temperature. 200 ml of 1 molar

discs and washed once with 0.1 M sodium borate buffer. IAB-oligonucleotide was prepared as described above and mixed with the membrane discs. 300 membrane discs were submerged in 2 ml of 0.1 M sodium borate buffer containing 1.0 mg of IAB-oligonucleotide, and the reaction was allowed to proceed at room temperature with constant agitation for 16 hours in the dark. The discs were then washed sequentially with 0.1 M sodium borate, SDS/FW. 1.2 micrograms of Bg5B oligonucleotide was bound per filter disc. The unreacted thiol groups were blocked with 50 mg/ml iodoacetamide in 0.1 M sodium borate pH = 8.3. The filters were then washed further with sodium borate and SDS/FW.

Lysis of bacteria and hybridization conditions:

1×10^8 Cells of Bacteroides gingivalis (Bg) were lysed in 100 µL of lysis solution at 19°C. The cell lysate was then heated in an 65 degree water bath for 10 minutes. Biotinylated probe was added to the lysate solution and to the diluent (GuSCN lysis solution) to a final concentration of 100 ng/mL, and 5 to 8 5-fold serial dilutions were made of the starting lysate. The solutions were incubated with either the derived nylon bead or the Nytran™ that had been covalently immobilized with 0.1 µg of respective oligonucleotide probe (capture probe) for 1 hour at ambient temperature with mild agitation. The solid supports were then washed once with the lysis and hybridization solution, once with FW, and once with SDS/FW. Streptavidin/HRP conjugate was added to a final concentration of 1 microgram/ml (based on streptavidin) in SDS/FW and incubated 10 to 15 minutes at ambient temperature with mild agitation. The beads and filters were then washed three times with SDS/FW and then once with CAP buffer. 4-methoxy-naphthol napthol substrate solution described above was added, and the reaction was allowed to proceed for 15 minutes at ambient temperature. The beads or filters were then quickly washed once with SDS/FW and then once with FW and allowed to air dry in the dark. The extent of hybridization is directly correlated with the density of the colored substrate deposited on the beads.

Example 1

Example 1 describes the selective modification and activation of the tethered 5'-amine of oligonucleotides with cyanuric chloride. It is shown that the derivitization of the oligonucleotide occurs only at the tethered amine.

The sequence UP9A, which either possessed a 5'-aminohexyl tail or did not possess a 5'-aminohexyl tail, was compared with respect to reactivity with cyanuric chloride. 50 ug of each type oligonucleotide was reacted in a 400 ul volume containing 0.15 M sodium borate pH = 8.3, 2 mg/ml cyanuric chloride (from 50 mg/ml freshly prepared stock in 100% acetonitrile). The reaction was allowed to occur at 19°C for 30 minutes. The reaction mixture was then analyzed by C-18 reverse phase HPLC utilizing a 5 to 45% acetonitrile gradient in TEA. The chromatographs from the respective reaction mixtures and both types of starting oligonucleotide are shown in Fig. 3.

The non-tailed oligonucleotide sequence UP9A is shown in panel A and elutes off the column at 9.025 minutes whereas the amine-tailed oligonucleotide sequence UP9A elutes at 9.205 minutes (panel B). The chromatograph in panel C shows that the non-tailed oligonucleotide sequence UP9A does not react with cyanuric chloride, as the oligonucleotide continues to be eluted at 9.005 minutes. In panel D, the amine-tailed oligonucleotide sequence UP9A reacts almost to completion with cyanuric chloride resulting in a dichlorotriazine derivitive that is eluted at 11.6 minutes, almost 2.5 minutes later than the UP9A amine-tailed starting material. The profiles indicate that only the UP9A sequence possessing the 5'-tethered amine reacted with cyanuric chloride demonstrating that cyanuric chloride reacted selectively with the amine and not with any of the sugars or bases present in the oligonucleotide. Therefore, it was shown that 5'-aminchexyl oligonucleotides are selectively activated with cyanuric chloride, resulting in a probe is immobilized only at the 5'-end onto a solid support.

Example 2

This example describes the derivation of nylon beads with several types of diamines and poly-(ethyleneimine), and then the subsequent attachment of 4,6-dichlorotriazine oligonucleotides. A comparison of the hybridization properties of the respective beads is also described.

200 bead batches were derived with either hexanediamine, Jeffamine EDR 148, 1,4-phenylenediamine, Jeffamine T3000, or poly(ethyleneimine) using the procedure described above relating to preparation of polymer-coated beads. Each diamine or triamine bead type contained between 200 nmoles to 2 umoles of amine and the poly(ethyleneimine) contained approximately 100 nmoles of amine.

solution containing the biotinylated signal oligonucleotides and one part in ten of whole blood. The solutions were then incubated for 30 minutes at ambient temperature with a Nytran™ discs or 2 nylon beads that had covalently immobilized 0.1 µg of BgI specific oligonucleotide probe (capture probe). The solid supports were then washed with SDS/FM at ambient temperature and incubated with 10 ng/ml of streptavidin/horseradish peroxidase (SA/HRP) conjugate in SDS/FW for 5 minutes at ambient temperature. The solid supports were then washed with SDS/FW, FW, and then the presence of peroxidase was determined by incubating the filter with the HRP substrate solution described above to form an insoluble product.

The results indicated that, in the 30 minute hybridization, 8×10^5 cells were detected using the nylon beads as solid supports whereas 4×10^6 cells were detected using Nytran™ solid supports. More importantly, the Nytran™ filters were significantly stained with lysed blood products whereas the nylon beads retained their starting color.

Example 5

Example 5 compares the solid supports formed from Nytran™ membranes and nylon beads in a sandwich assay format in which a target nucleic acid sequence is sequestered and then detected using a chemiluminescence assay format.

3 M GnSCN lysis solution was used to lyse 1×10^8 cells of Actinobacilius actinomycetemcomitans (Aa), Bacteroides gingivalis (Bg), Bacteroides intermedius (Bi), Eikenella corrodens (Ec), Fusobacterium nucleatum (Fn), and Wolinella recta (Wr) in 100 microliter volumes at 19°C. The lysate was then heated to 65°C for 5 minutes. A biotinylated 24-mer oligonucleotide probe complementary to conserved regions of bacterial 16s rRNA (signal probe) was added to a final concentration of 100 nanograms per ml.

5-fold serial dilutions of the lysates were made using diluents in 3 M GuSCN lysing and hybridization solution containing the biotinylated signal oligonucleotides and 1×10^8 total cells of Aa, Bi, Ek, Fn, and Wr. The solutions were then incubated for 30 minutes at ambient temperature with Nytran™ discs or 2 nylon beads that had covalently immobilized 0.1 µg of BgI specific oligonucleotide probe (capture probe). The solid supports were washed with SDS/FW at ambient temperature following by washing with 0.5% Tween 20, 1 mM MgCl2, 0.01 M Tris-HCl pH 8.0 (APB) and then incubated with 0.4 ug/ml of streptavidin/alkaline phosphatase (SA/AP) conjugate in APB for 5 minutes at ambient temperature. The solid supports were washed 5 times with APB, TMNZ, and then the presence of alkaline phosphatase was determined by incubating either the Nytran™ filters or the nylon beads with 200 microliters of Lumigen (from Lumigen, Inc., Detroit, MI) in 5mm x 40 mm polypropylene tubes. The results are shown in the table below.

	Chemiluminescent Signal	
Cell number:	Nytran™ solid supports:	Nylon beads:
1×10^8	off scale	off scale
2×10^7	1600	off scale
4×10^6	1750	1650
8×10^5	1700	680
1.6×10^5	1600	320
3.2×10^4	1800	260
6.4×10^3	1800	210
control	1700	200

Therefore, the results indicate that, in the 30 minute hybridization, 3×10^4 cells were detected using the nylon beads as solid supports whereas only 1×10^8 cells were detected using the Nytran™ solid supports. This approximately 10,000-fold difference in the lower level of detection of the target was due to the severe background of nonspecific binding of the alkaline phosphatase to the Nytran™ filters. The nylon beads, therefore, allowed the sensitive detection of Bg 16s rRNA using a chemiluminescence based signal system.

Example 6

SEQUENCE LISTING FOR EUROPEAN PATENT APPLN. 90309671.7

SEQ ID NO: 1
SEQUENCE TYPE: Nucleotide
SEQUENCE LENGTH: 24
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: synthetic oligonucleotide
CAATACTCGTATCGCCCGTTATTC

SEQ ID NO: 2
SEQUENCE TYPE: Nucleotide
SEQUENCE LENGTH: 24
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: synthetic oligonucleotide
ACCCATCTCTGACTTCTTCTTCGG

SEQ ID NO: 3
SEQUENCE TYPE: Nucleotide
SEQUENCE LENGTH: 24
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: synthetic oligonucleotide
TACTCGTATCGCCCGTTATTCCCG

SEQ ID NO: 7
SEQUENCE TYPE: Nucleotide
SEQUENCE LENGTH: 20
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: synthetic oligonucleotide
CTGCTGCCTCCCGTAGGAGT

SEQ ID NO: 8
SEQUENCE TYPE: Nucleotide
SEQUENCE LENGTH: 18
STRANDEDNESS: single
TOPOLOGY: linear
MOLECULE TYPE: synthetic oligonucleotide
GTATTACCGCGGCTGCTG

Claims

1. A composition comprising an oligonucleotide covalently attached to a polymer-coated bead.
2. The composition according to claim 1 wherein the oligonucleotide is activated with a monofunctional or multifunctional reagent.
3. The composition according to claim 2 wherein the reagent is homotrifunctional.
4. The composition according to claim 3 wherein the reagent is cyanuric chloride.
5. The composition acording to claim 1 wherein the polymer is an amine-containing polymer.
6. The composition according to claim 5 wherein the polymer is activated with cyanuric chloride.
7. The composition according to claim 6 wherein the polymer-coated bead is nylon and spherical in shape.
8. The composition according to claim 7 wherein the polymer-coated bead has a diameter in the range from about 0.01 inch to about 0.5 inch.
9. The composition according to claim 8 wherein the polymer-coated bead has a diameter from about 0.06 inch to about 0.09 inch.
10. The composition according to claim 9 wherein the polymer-coated bead has a diameter of about 0.09 inch.
11. The composition according to claim 10 wherein the oligonucleotide is activated through a 5'- or 3'-tethered amine.

31. The process according to claim 30 wherein the solid support is a bead.

32. The process according to claim 31 wherein the alkylating agent is trialkyloxonium salt.

33. The process according to claim 32 wherein the polymer is selected from the group consisting of poly-(ethyleneimine), polyvinylamine, and polyallylamine.

34. The process according to claim 33 wherein the reagent is homotrifunctional.

35. The process according to claim 34 wherein the reagent is cyanuric chloride.

36. The process according to claim 35 wherein the blocking agent is succinic anhydride.

37. A process for nucleic acid detection comprising:
contacting a composition of claim 1 with a target nucleic acid under suitable conditions for hybridization; and
detecting the hybridized product.

38. The process according to claim 37 wherein the process occurs in a microtiter well.

39. The process according to claim 37 wherein the process occurs in a flow-through tube.

40. The process according to claim 37 wherein the composition comprising an oligonucleotide covalently attached to a polymer-coated bead is attached to a perforation in a dipstick.

41. A process for activating an oligonucleotide comprising treating the oligonucleotide with cyanuric chloride.

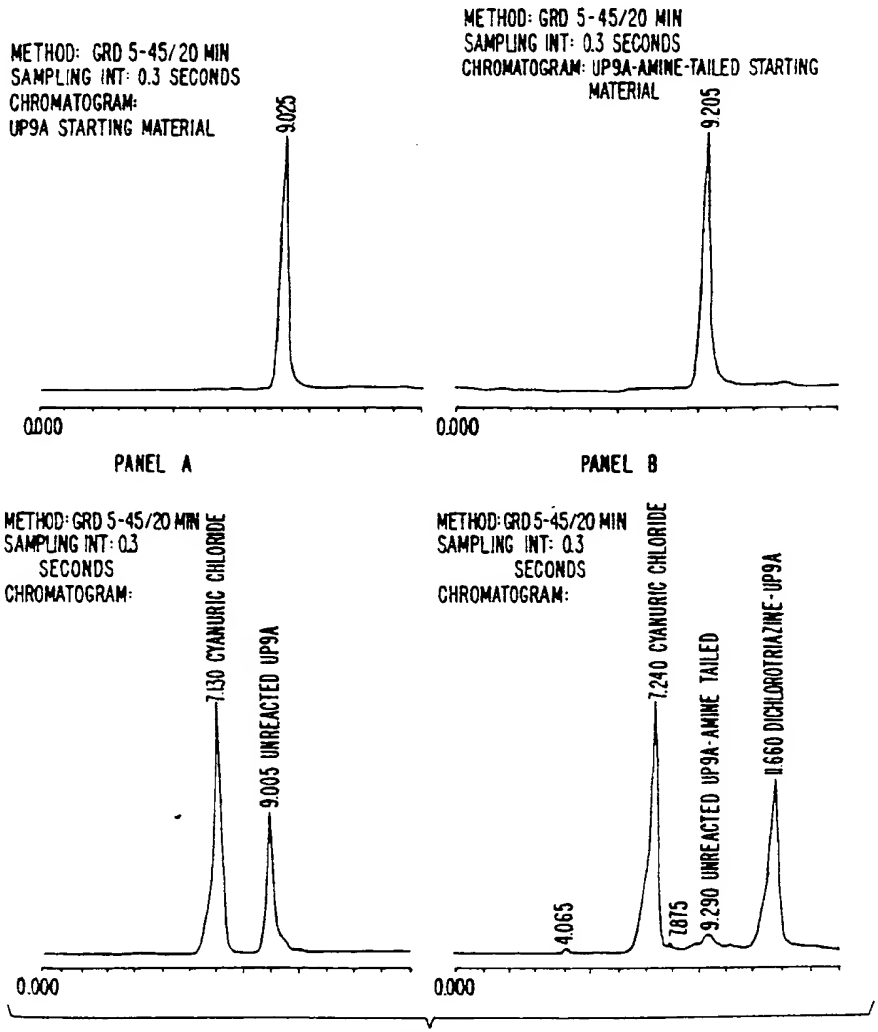
METHOD: GRD 5-45/20 MIN
SAMPLING INT: 0.3 SECONDS
CHROMATOGRAM:
UP9A STARTING MATERIAL
9.025
0.000
PANEL A
METHOD: GRD 5-45/20 MIN
SAMPLING INT: 0.3 SECONDS
CHROMATOGRAM: UP9A-AMINE-TAILED STARTING MATERIAL
9.205
0.000
PANEL B
METHOD: GRD 5-45/20 MIN
SAMPLING INT: 0.3 SECONDS
CHROMATOGRAM:
7.130 CYANURIC CHLORIDE
9.005 UNREACTED UP9A
0.000
METHOD: GRD 5-45/20 MIN
SAMPLING INT: 0.3 SECONDS
CHROMATOGRAM:
7.240 CYANURIC CHLORIDE
4.065
7.875
9.290 UNREACTED UP9A-AMINE TAILED
11.660 DICHLOROTRIAZINE-UP9A
0.000

FIG._3.